J. Dairy Sci. 96:378–383
http://dx.doi.org/10.3168/jds.2012-5811

Short communication: Effect of heat stress during the dry period on gene expression in mammary tissue and peripheral blood mononuclear cells

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ABSTRACT

Heat stress (HT) during the dry period compromises mammary gland development, decreases future milk production, and impairs the immune status of dairy cows. Our objective was to evaluate the effect of cooling HT cows during the dry period on gene expression of the mammary gland and peripheral blood mononuclear cells (PBMC). Cows were dried off 46 d before their expected calving and assigned to 2 treatments, HT or cooling (CL). Cows in the CL group were cooled with sprinklers and fans whereas HT cows were not. After parturition, all cows were housed in a freestall barn with cooling. The PBMC were isolated at dry-off and at −20, 2, and 20 d relative to calving from a subset of cows (HT, n = 9; CL, n = 10), and mammary biopsies were taken at the same intervals (HT, n = 7; CL, n = 6) for RNA extraction. Gene expression was assessed using a custom multiplex gene expression assay based on traditional reverse transcription-PCR. Genes involved in prolactin (PRL) signaling [PRL receptor long form, PRL receptor short form, suppressor of cytokine signaling (*SOCS*)*2*, *SOCS3*, *IGF2*, IGF binding protein 5, and cyclin D1], fatty acid metabolism (acetyl-CoA carboxylase α (*ACACA*) and lipoprotein lipase (*LPL*)], and *IGF1* were evaluated in mammary tissue, and genes related to fatty acid metabolism [*ACACA*, fatty acid synthase (*FASN*), and *LPL*], cytokine production [*IL6*, *IL8*, and tumor necrosis factor (*TNF*)], and *IGF1* were evaluated in PBMC. No differences were observed in PRL signaling or fatty acid metabolism gene expression in the mammary gland. In PBMC, HT cows had greater mRNA expression of *IGF1* and *TNF* during the transition period relative to CL and upregulated *IL8* and downregulated *FASN* mRNA expression at 2 d relative to calving. We conclude that cooling HT cows during the dry period alters expression of genes involved in cytokine production and lipid metabolism in PBMC.

Key words: dry period, heat stress, peripheral blood mononuclear cells, mammary gland

Short Communication

Environmental modification during the dry period such as heat stress (**HT**) and photoperiod exert dramatic effects on dairy cows. For example, compared with long-day photoperiod (16 h light: 8 h dark, **LDPP**), short-day photoperiod (8 h light: 16 h dark, **SDPP**) upregulates mammary gland remodeling before parturition (Wall et al., 2005) and increases milk yield of cows in the next lactation (Auchtung et al., 2005). Similar to SDPP, cooling also enhances mammary growth during the dry period and improves lactation performance after parturition (Tao et al., 2011). Prolactin (**PRL**) plays important roles in bovine lactogenesis and mammogenesis (Tucker, 2000), and evidence indicates that the altered PRL signaling mediates photoperiodic effects on mammary gland development during the dry period. Specifically, SDPP decreases the concentration of PRL in blood but increases gene expression of PRL receptor (***PRLR***) in mammary tissue relative to LDPP (Auchtung et al., 2005). The cellular mechanism of the compromised mammary gland development by HT during the dry period is still unknown and is thought to be associated with modified PRL signaling (Tao et al., 2011). However, data related to gene expression in the PRL signaling of mammary gland in HT or cooled (**CL**) dry cows are still not available. In addition, the effect of cooling HT dry cows on gene expression involved in FA metabolism in the mammary gland has yet to be evaluated.

Heat stress also influences the animal's immune function during the dry period. Relative to CL cows, HT animals when dry have decreased proliferation of peripheral blood mononuclear cells (**PBMC**) and decreased production of tumor necrosis factor (**TNF**)-α in response to mitogen in vitro during the transition period (do Amaral et al., 2010). However, the effect of dry period HT on endogenous inflammatory cytokine gene expression of PBMC during the transition period

Received June 5, 2012.
Accepted October 4, 2012.
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has never been evaluated. In addition, gene expression data for *IGF1* and enzymes involved in the FA metabolism of PBMC in HT dry cows are not available. We hypothesized that HT during the dry period suppresses PRL signaling and FA metabolism in the mammary gland and alters inflammatory cytokine gene expression of PBMC. Therefore, our objective was to evaluate the effect of HT and CL during the dry period on gene expression of the mammary gland and PBMC of dairy cows during the transition period.

Cows used in the current study were from a subset of animals in a larger study, and details of treatment and management of animals were reported previously (Tao et al., 2011). University of Florida Institute of Food and Agricultural Sciences Animal Research Committee approved all procedures. Briefly, cows were dried off approximately 46 d before expected calving date and randomly assigned to 2 treatments, HT or CL. During the dry period, CL cows were cooled with shade, sprinklers, and fans, and HT cows were provided with shade only. After parturition, all cows were managed as a single group. Mammary biopsies were collected from a subset of animals (HT, n = 7; CL, n = 6) at dry-off and at −20, 2, and 20 d relative to calving, and PBMC were isolated at the same intervals (HT, n = 9; CL, n = 10). The mammary biopsy procedure was described previously (Tao et al., 2011), and PBMC isolation was performed based on methods reported by do Amaral et al. (2010).

The RNeasy Midi kit (Qiagen, Valencia, CA) was used for total RNA extraction of the mammary tissues and PBMC, following the manufacturer's protocol with on-column DNase treatment. Quality of RNA was assessed using the Agilent 2100 Bioanalyzer with RNA 6000 Nano LabChip kits (Agilent Technologies, Palo Alto, CA) and RNA concentration was determined using the ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). Gene expression was evaluated by a custom 22-gene multiplex expression assay on the GeXP Genetic Analysis System and GeXP Start Kit (Beckman Coulter Inc., Brea, CA), which is based on traditional reverse transcription-PCR. Assay optimization, generation of standard curves, and assay procedures were performed based on manufacturer's instructions. Details can be found in Connor et al. (2010). Briefly, total RNA (100 ng) was reverse transcribed with pooled gene-specific reverse primers containing a universal sequence attached at the 5′ end (Table 1). The KanR RNA (2.5 pg/reaction) provided in the GeXP Start Kit was added as an internal normalization control. Negative control reactions in the absence of reverse transcriptase were also performed on each sample to confirm the absence of contaminating genomic DNA. Reaction conditions were 48°C for 1 min, 42°C for 60 min, 95°C for 5 min, and then holding at 4°C. Reactions for subsequent PCR included 25 m*M* $MgCl_2$, pooled forward primers, cDNA, PCR buffer, and Thermo-Start Taq DNA polymerase (Thermo Scientific, Waltham, MA), according to the manufacturer's instructions and optimized conditions. The PCR reaction conditions were 95°C for 10 min, 94°C for 30 s, 55°C for 30 s, and 70°C for 1 min for 35 cycles. The PCR products were diluted in sample loading solution provided in the GeXP Start Kit plus DNA size standard 400 and analyzed by GenomeLab GeXP Genetic Analysis System (Beckman Coulter Inc.). Data were exported to the eXpress Profiler Program (Beckman Coulter Inc.), calculated as area under the curve, and normalized to KanR RNA. The final data were normalized to the most stably expressed reference genes as determined by the GeNorm program (http://medgen.ugent.be/~jvdesomp/genorm/) using the GeneQuant Tool (Beckman Coulter Inc.) and reported as the value relative to the geometric mean expression of the reference genes. For PBMC RNA, gene expression was normalized to that of β-2 microglobulin and *GAPDH* (GeNorm M-value = 0.33) and for mammary RNA, expression was normalized to that of ATP synthase, β-2 microglobulin, and hypoxanthine phosphoribosyltransferase 1 (M-value <0.70).

Gene expression data were analyzed using repeated measures of PROC MIXED of SAS 9.2 (SAS Institute, Cary, NC), and least squares means ± standard errors of the means are presented. The statistical model included treatment, time, and treatment × time interaction, with cow within treatment as a random effect. The data from the samples taken at dry-off were considered as covariates and included in the SAS model. If a treatment × time effect was observed, the treatment effect at individual time points was obtained by the SLICE function in SAS models. Data were considered significant if $P < 0.05$ or a trend if $P < 0.15$.

Tao et al. (2011) reported the details of the physiological responses of the cows within the experiment. Briefly, during the dry period, the temperature–humidity index ranged from 72 to 81 throughout a day and averaged 76.6 for all cows. Compared with CL cows, HT cows had elevated body temperatures during the dry period, averaging 38.98 and 39.34°C, respectively. Relative to CL cows, HT cows had a reduced mammary cell proliferation rate at −20 d relative to calving but not at other time points. We observed no differences between treatments for the mammary cell apoptotic rate at any time point in the transition period. Compared with cooling, heat stress during the dry period also increased the PRL concentration in plasma .

In mammary tissue, no treatment or treatment × time effects were observed in mRNA expression for